APPLICATION NOTE

AX/AX R with NSPARC Confocal based Super Resolution Microscope

Quantitative Analysis of Mitochondrial Microstructure by Super-Resolution Imaging

Mitochondria are intracellular organelles that take on the essential functions for life activities such as ATP production and apoptosis. Their form changes dynamically and they repeatedly divide and fuse within cells. Also, mitochondria consist of double membranes comprising an outer membrane and inner membrane, the inner membrane having a structure called a crista that greatly increases surface area, allowing important chemical reactions to occur. There is a correlation between the morphology or structure of mitochondria and cell viability, and research focusing on the relationship between mitochondrial morphology and structure and human disease is currently being developed.

This application note introduces examples of mitochondrial observation using the AX/AX R with NSPARC (Nikon SPatial ARray Confocal) confocal-based super resolution microscope. Cristae with fine structures and dynamic morphological changes were clearly observed by combining NSPARC with a high-speed resonant scanner. NSPARC, which can acquire super-resolution images easily with the same operability as a conventional confocal microscope, can be applied not only to academic research, but to medical application fields such as medical treatments and drug discovery screening.

Keywords: Confocal Microscope, Super-Resolution Imaging, Live Cell imaging, Mitochondria

Overview of Experiment 1

Mitochondria move around dynamically in cells and repeatedly divide and fuse. Since these events happen in seconds, high-speed super-resolution imaging is essential to accurately capture the dynamics of mitochondria. In time-lapse imaging, phototoxicity and photobleaching of the fluorescent probe are also major problems.

In this experiment, the mitochondria in HeLa cells cultured for 2 days at 37°C and 5% CO_2 were stained with MitoTracker™ Green FM, and live cell imaging was performed with an AX/AX R with NSPARC confocal-based super resolution microscope. A resonant scanner was used for high-speed imaging and to reduce photo damage and photobleaching.

Results

A comparison of the confocal image, and super-resolution image acquired with NSPARC is shown in Fig. 1. Microstructure cristae that were not clearly captured in the confocal image were clearly captured in the NSPARC image. In addition to the outer shape of mitochondria, quantification of the crista structure, which has attracted attention for its correlation with mitochondrial activity, has also become possible.

Subsequently, time-lapse imaging of mitochondria was performed using NSPARC (Fig. 2). Since the morphology of mitochondria actively changes, slow acquisition speed will lead to a blurred image, which results in a decreased resolution. The time-lapse imaging of this experiment (1024 x 512 pixels, 3.8 frames/sec, 3 sec. intervals) was performed using a high-speed resonant scanner. Figure 2 shows part of this movie. Minute morphological changes at the moment of mitochondrial fission and fusion, which were difficult to capture with a conventional confocal microscope, can now be easily observed.

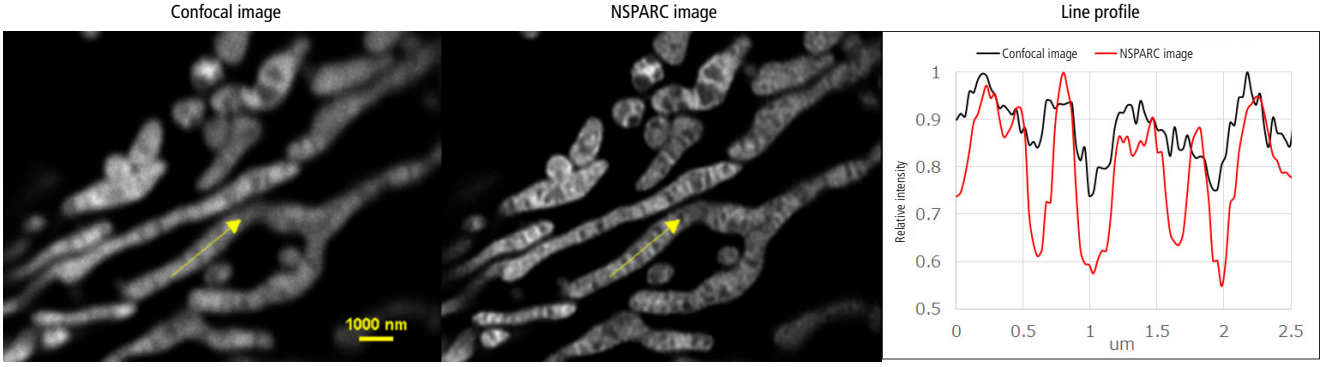


Fig. 1: Comparison of confocal and NSPARC images

HeLa cells stained with MitoTracker™ Green FM: confocal image (left) and NSPARC image (right): Cristae were clearly observed in NSPARC image. A CFI Plan Apochromat Lambda D 60X Oil objective was used.

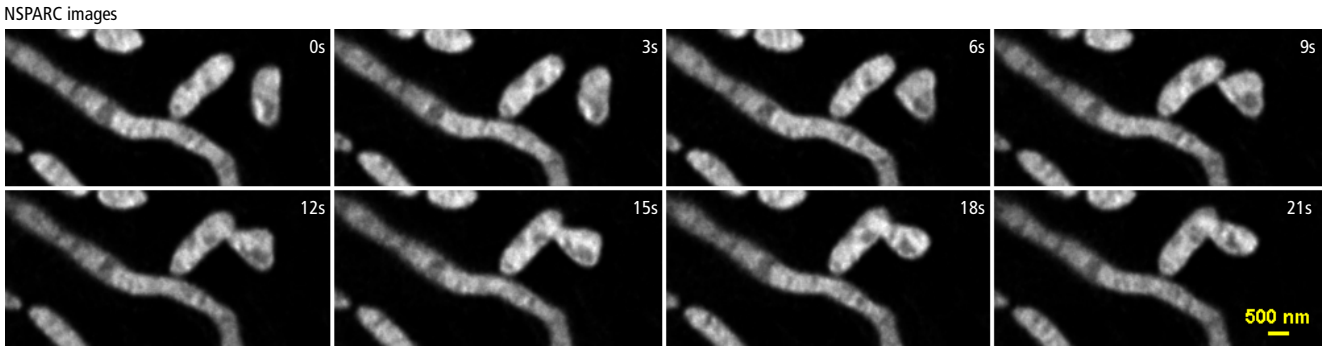


Fig. 2: Super-resolution images using resonant scanning

Extracted and displayed time-lapse images of 3.8 frames/sec, at 3 sec. intervals. The state of mitochondria fusion during transformation is captured in detail. A CFI Plan Apochromat Lambda D 60X Oil objective was used.

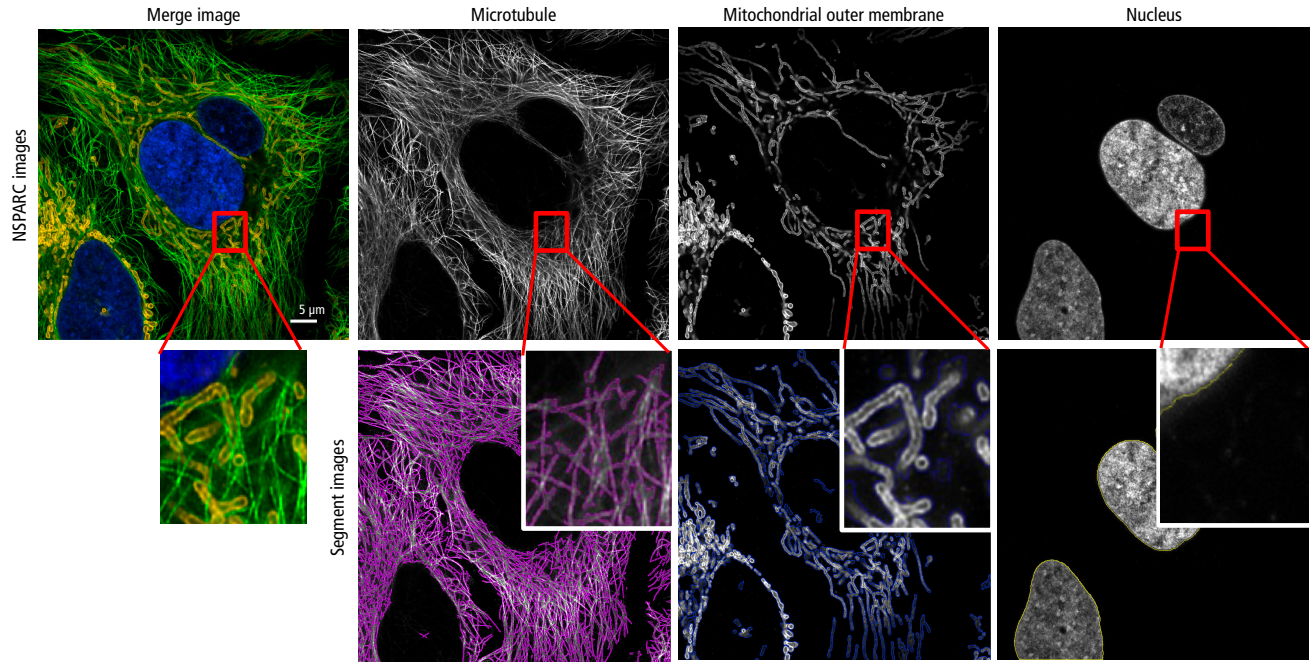
Overview of Experiment 2

Observation of a fixed IHC-stained sample was performed. Microtubules of HeLa cells were stained with Alexa Fluor™ 488, the mitochondrial outer membrane was stained with Alexa Fluor™ 568, and the nucleus was stained with DAPI. Line sequential multicolor imaging was performed with excitation wavelengths of 405 nm, 488 nm, and 561 nm. Quantification was then performed on the acquired images using the NIS-Elements Analysis Module (GA3).

Results

In the NSPARC images, the contrast of both microtubules and the mitochondrial outer membrane was increased, and fine structures were more clearly acquired (Fig. 3). As a result, accurate numerization and localization analysis are possible even when quantification is performed. When comparing the quantitative results of each staining, the microtubule region had a total extension distance about 4 times and an area 1.5 times that of the mitochondrion. On the other hand, in terms of width (thickness), the mitochondrial region was 3.6 times that of the microtubules.

The quantification described above is becoming essential in analyses that utilize imaging. More accurate and detailed image analysis than ever before becomes possible by combining NSPARC super-resolution imaging and NIS-Elements analysis, and its application to drug discovery and development, which require high reproducibility, is eagerly anticipated.



Measurement item	Microtubule	Mitochondrion
Length (total): µm	4387.23	1149.72
Width (average): µm	0.16	0.59
Total area: µm^2	1173.18	755.91

Measurement item	Nucleus
Count	3
Area (average): µm^2	209.97
Diameter (average): µm	19.63

Fig. 3: Observation of immunostained sample

IHC-stained sample using fixed HeLa cells: microtubules were stained with Anti-alpha Tubulin antibody (abcam #ab6160) and the mitochondrial outer membrane was stained with TOMM20 (abcam #ab78547), and Alexa Fluor™ 488 (Invitrogen #A-11006) and Alexa Fluor™ 568 (Invitrogen #A-11011) were used as secondary antibodies for microtubules and mitochondria, respectively. A CFI Plan Apochromat Lambda D 60X Oil objective was used.
Upper row = images of each stain, Lower row = segment image, Table = NIS-Elements quantitative results

Product Information

AX/AX R with NSPARC Confocal based Super Resolution Microscope

The NSPARC super resolution detector, comprising an array of 25 detectors, achieves resolution superior to conventional confocal imaging with a high SN ratio.

CFI Plan Apochromat Lambda D 60X Oil

Boasts excellent image flatness and achieves a uniform image right to the edge of its large field of view of FOV25. Suitable for multicolor imaging by chromatic aberration correction over a wide wavelength range.
NA = 1.42, WD = 0.15mm